Fish Bioassay and Toxin Induction Experiments for Research on *Pfiesteria piscicida* and Other Toxic Dinoflagellates: Workshop Summary

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In late January 2000, the Centers for Disease Control and Prevention sponsored a workshop to discuss standardizing the laboratory materials and methods used for *in vivo* fish bioassays and toxin induction experiments. Representatives from six laboratories using these assays to conduct research on *Pfiesteria piscicida* Steidinger & Burkholder, similar organisms (i.e., members of the toxic *Pfiesteria* complex) or their toxins were invited to attend. The workshop objectives were *a*) to discuss the need for uniform quality assurance for fish bioassays and toxin induction, *b*) to encourage publishing the relevant materials and methods in the literature, *c*) to foster communication among the laboratories conducting this work, and *d*) to respond to requests from state health and environmental protection agencies for guidance in interpreting the results from fish bioassays conducted in different laboratories. To facilitate discussion at the workshop, researchers conducting *Pfiesteria* research completed a detailed questionnaire in advance about fish bioassays and toxin production assays. Workshop participants discussed experimental factors that might influence the reproducibility or interpretation of fish bioassays and toxin-induction experiments. The experimental factors were categorized into physical, chemical, and biological parameters. In addition, participants ranked experimental factors by their relative importance in conducting these assays as *a*) factors that are critically important and should be maintained within a recommended range, *b*) factors that are important in conducting the assays but that may be variable among laboratories or within experiments and whose values should be recorded and reported by investigators, and *c*) factors of unknown importance that should be considered important research questions. This article summarizes results obtained from the questionnaire and workshop discussions. *Key words*: fish bioassays, laboratory aquariums, *Pfiesteria piscicida,* toxin-induction assays. — *Environ Health Perspect* 109(suppl 5):769–774 (2001).
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Background

The presence of *Pfiesteria piscicida* Steidinger & Burkholder in estuarine waters has prompted concerns about adverse effects on human health, fish health, and estuarine ecology (*1–4*). Estuaries are complex environments affected by many factors, including meteorologic conditions, land use patterns, and seasonal changes. To understand how the presence of *P. piscicida* or similar organisms and any toxins they may produce affect other organisms, investigators have used toxin-induction experiments to produce toxic material for identification and characterization and fish bioassays to assess toxicity (*4–9*).

The toxin-induction experiment is performed for the purpose of producing relatively high densities of toxin-producing organisms in various volumes of water. Live fish are added to the aquarium (or culture vessel) as a means of stimulating toxin production, even though fish death is not an experimental end point. The aquarium is then observed. As fish die, they are removed and replaced. Successful toxin production presupposes that the investigator has available a supply of organisms capable of producing toxin.

Different from the toxin-induction experiment, the fish bioassay has the experimental end point of fish death. It is also used to detect the presence of other ichthyotoxic dinoflagellates that have not been recognized in estuaries. These organisms, once verified as ichthyotoxic and formally described, could be added to known toxic *Pfiesteria* complex (TPC) species. The end point may be a binary indicator (the sample contains or does not contain a toxin or toxin-producing organism) or a more quantitative measure of toxicity (e.g., the concentration of organisms necessary to kill all newly exposed fish within a certain period).

These *in vivo* laboratory assays represent an attempt to reproduce the critical components of estuarine conditions, thereby providing investigators with the opportunity to understand the impact of toxins on the biological, chemical, and physical processes that affect fish health. In addition to the basic scientific interest in dinoflagellate ecology and behavior (including toxin production), several state public health and environmental protection agencies use the results of fish bioassays to make decisions about public use of estuarine waters for seafood harvesting and recreation. It is important for these agencies to have access to appropriately interpreted scientific data that demonstrate not only whether the organisms are present but whether they are producing toxins as well. The release of incomplete information (i.e., the microscopic analyses confirmed that *Pfiesteria* was present, but the tests for toxicity were not done or were not yet completed) or inconsistent information (i.e., one laboratory confirms the presence of the organism and toxicity, whereas another confirms the presence but without toxicity) makes it impossible for public health officials to accurately assess the potential public health impact from the presence of *Pfiesteria* in estuarine waters.

Many laboratories must rely on other laboratories to produce toxins for use in their analytical, biochemical, and molecular research activities. Misidentification of the organisms used to produce the toxic material and inconsistent methods for toxin induction may frustrate attempts to identify and characterize toxins produced by this group of dinoflagellates. Thus, it is critical that researchers working with the fish bioassays and toxin-induction experiments develop a consensus and eventually a standardized protocol that includes materials and methods for conducting and interpreting fish bioassays and toxin-induction experiments.

Because the bioassays are still being refined, a standardized protocol cannot be established; however, good laboratory practices that include recording physical, chemical, and biological parameters that were the subject of discussions at the workshop can be used over time to develop a standardized protocol. In the immediate future, however, monitoring and recording these parameters may be helpful when determining why two different laboratories may provide different interpretations after analyzing similar samples, or even portions of a split sample.

Workshop

In late January 2000, the Centers for Disease Control and Prevention sponsored a workshop to discuss standardizing the

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laboratory materials and methods used for *in vivo* fish bioassays and toxin-induction experiments. Representatives from six laboratories using these assays to conduct research on *P. piscicida*, similar organisms (i.e., members of the TPC), or their toxins were invited to attend. Representatives from five of these laboratories participated in the workshop. Workshop objectives were *a*) to discuss the need for uniform quality assurance methods for fish bioassays and toxin-induction experiments, *b*) to encourage publishing relevant materials and methods in the literature, *c*) to foster communication among the laboratories conducting this work, and *d*) to respond to requests from state health and environmental protection agencies for guidance in interpreting the results from fish bioassays conducted in different laboratories.

Questionnaire and Workshop Discussion

Investigators at the National Center for Environmental Health developed and distributed a questionnaire requesting details about the materials and methods, including physical parameters, test organism species, and the use of positive and negative controls used by each laboratory to conduct fish bioassays and toxin-induction experiments. A total of nine laboratories were sent questionnaires (Table 1). Questionnaire responses and comments from 13 individuals (9 of 9 laboratories) were compiled to form the basis of discussions at the workshop.

Table 1. Laboratories that completed the fish bioassay and toxin induction questionnaire developed by the Centers for Disease Control and Prevention.

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Workshop participants discussed experimental factors that might influence the reproducibility or interpretation of fish bioassays and toxin-induction experiments. The experimental factors were categorized into physical, chemical, and biological parameters. In addition, participants ranked experimental factors by their relative importance in conducting these assays as *a*) factors that are important to system reproducibility and should be maintained within a recommended range, *b*) factors that are important in conducting the assays but that may be variable among laboratories or within experiments and whose values should be recorded and reported by investigators, and *c*) factors of unknown importance that should be considered important research questions.

Results

Identifying criteria to monitor for quality assurance in these assays was a central concept in the workshop. Quality assurance is the entire process of monitoring a system for reproducibility and reliability. A system must be well understood so that the criteria describing a correct operating system are monitored, and corrective action can be taken when those criteria are not met.

Quality Assurance/Quality Control

Quality control is part of the quality assurance process and constitutes the actual record of performance of the system and of actions taken when system performance standards are not met. Positive and negative quality control materials are used as part of this process to assess performance of a system. These materials will have a matrix similar to the known material so that unknown specimens and quality control samples have similar responses. In addition, these materials have long-term stability and minimal variance. A positive quality control material will yield a response similar to the known material under study, whereas a negative quality control material will not give a response.

When conducting analyses that include quality assurance methods, frequent evaluation of quality control materials is critical so that performance problems can be quickly identified and resolved. When possible, shared or standardized material should be used among laboratories conducting similar work. Analysis of shared or standardized material allows for evaluation of laboratory performance and reliability. In addition, the sensitivity of the assay being conducted can be evaluated over time.

Ideally, positive and negative control materials should be used in conducting experiments. Because a toxin may be produced by this experiment, a material with similar biological effect would be first choice as a positive quality control material. The identity of the *Pfiesteria* toxin and its mode of action are still unknown, and it has not been isolated. This material, therefore, cannot be used as a positive control material. Other toxins could potentially be used, but it is unknown if they would affect fish in the same way as the *Pfiesteria* toxin. Also, the addition of a toxin would not mimic the environmental conditions that would be otherwise present in the experimental system from the use of the organism.

As *Pfiesteria* toxin is unavailable, dinoflagellate organisms were discussed as potential sources of positive and negative control materials for the assays. Dinoflagellate organisms cultured in marine or estuarine environments (saltwater) would have a similar matrix to a collected water sample and produce similar conditions as the *Pfiesteria* organism, which would satisfy part of the requirements for quality control materials. *P. piscicida* or a member of the TPC known to produce toxin would be an appropriate positive control. As described earlier, however, these materials need to have long-term stability, and researchers have discovered that over time *P. piscicida* loses its ability to produce toxin. Possible negative quality control organisms include nontoxic *P. piscicida* or other nontoxic *Pfiesteria* species.

Ultimately, the goal of quality assurance and quality control is that the variability of the mean value of repeated measurements is minimized (i.e., the investigator controls as many parameters as possible so that outcomes can be reproduced within a given set of conditions). The fish bioassay and toxin-induction experiments are dynamic biological systems that attempt to recreate complex environmental conditions, the specifics of which are still largely unknown. Given that the negative controls in laboratory experiments using *Pfiesteria* are most likely to be nontoxic strains of the organism itself and that no positive controls are currently available, it is critical that all investigators carefully record not only experimental results but also as much as possible about the conditions under which their experiments are conducted.

Because of the difficulties associated with conducting high-quality laboratory research with *P. piscicida* and similar organisms, workshop participants and questionnaire respondents were asked to identify any experimental parameters (physical, chemical, or biological) that should be either a specified value or within a specified range of values. The results of these discussions are presented in the discussion below and in Tables 2, 3, and 4..

Physical Parameters

The experimental variables describing physical parameters in these assays are listed in

Table 2. Physical parameters for conducting fish bioassays and toxin-induction experiments applicable to research involving *P. piscicida* and TPC organisms and the toxins they produce.[a]

Physical parameter	Specification or recommended range of values	Frequency of measurement and recording
Vessel type	Aquaria: 10 gallon Flask: 500 mL	Record for experiment Record for experiment
Water	Instant Ocean[b]	Record for experiment
Temperature	20–25°C	Daily
Salinity	0–35 PSU	Daily
Light cycling	14 hr light/day	Record for experiment
Light intensity	30 to 80 micro-Einsteins	Record for experiment

[a]The parameters presented are those with specifications or recommended ranges of values. Also included are the recommended frequencies for measuring the parameter and recording the value, if applicable. [b]Aquarium Systems, Mentor, OH, USA.

Table 3. Chemical parameters for conducting fish bioassays and toxin induction experiments applicable to research involving *P. piscicida* and TPC organisms and the toxins they produce.[a]

Chemical parameter	Specification or recommended range of values	Frequency of measurement and recording
Nitrate/nitrite	0 to <20 ppm	Initially, then every 3–4 days (daily if fish behave abnormally), and at completion of experiment
Ammonia	0.0–0.5 ppm	Initially, then every 3–4 days (daily if fish behave abnormally), and at completion of experiment
Dissolved oxygen	>5 ppm	Twice daily
pH	7.8–8.4	Daily
Antibiotics	Not recommended	If used, record observations
Fish food	Standard fish diet	Record type

[a]The parameters presented are those with specifications or recommended ranges of values. Also included are the recommended frequencies for measuring the parameter and recording the value, if applicable. More information about specific chemical parameters can be found in Luna (*21*).

Table 2. Variables consist of materials and conditions used in the experiments. Recommended ranges included the use of artificial seawater (Instant Ocean, Aquarium Systems, Mentor, OH, USA) and light cycling and intensity (14 hr of light per day and 30–80 microeinstein intensity); reportable values were salinity (salinity may vary in estuarine environments from 0 to 35 psu), water temperature (20–25°C), and type of vessel (aquaria or culture flasks).

The choice between using aquaria and using culture flasks is largely dictated by specific experimental objectives and considerations about laboratory safety. Open aquaria should be used only in an enclosed area with components of biohazard Biosafety Level 3 containment (personal protective equipment/chemical-fume hoods/biological isolation cabinets) to limit aerosol generation and prevent human exposure. Culture flasks are capped vessels and do not present an aerosol toxicity hazard when closed; however, they must be handled in chemical safety hoods or biological-safety cabinets to minimize contamination and to avoid human exposure to aerosols when they are opened. They must be opened periodically to perform chemical tests on the contents, to add or remove fish, and to perform an air exchange.

Water temperature influences the morphology and life stage transitions of *P. piscicida* and may do so within relatively short periods (i.e., within hours) (*6,7*). The recommended range of 20–25°C is environmentally appropriate, as it corresponds to typical spring and summer temperatures in the estuarine waters where the organism is found, and it is within the range of temperatures that correspond to fish kills attributed to *P. piscicida* (*8*). Researchers may wish to vary temperatures when environmental conditions at the time of sample collection are outside of this range. Varying temperature to examine effects on toxin production should be recorded and reported.

P. piscicida appears to grow better in an estuarine environment with higher salinity (15 psu) than do other dinoflagellates (*9*); however, it can survive and multiply in waters with a wide range (0–35 psu) of salinities (*8*). The values presented in Table 2 represent the range of salinity values (including seasonal and meteorologically related variability) observed in the estuarine waters of the Carolinas, Virginia, and the Chesapeake Bay region in Maryland and Virginia.

For many dinoflagellates, cell division is governed by the number of hours of light in a 24-hr period (i.e., the light cycle). Species in which the reproductive cycle is fully described belong to one of two classes: those in which cell division is triggered by the onset of darkness and those in which cell division is triggered by the onset of light. Although the influence of light, both in terms of light cycle and light intensity, on the life cycle of the TPC is unknown, researchers at the workshop believe it may be an important factor in the proliferation of these organisms (*9*).

Chemical Parameters

Measurable chemical parameters [e.g., dissolved oxygen (DO) and nutrients] (Table 3) may play an important role in triggering the production of toxins by TPC organisms in the environment. Values for these parameters should be included when reporting experimental results, both to monitor experimental conditions and possibly to provide insight about those environmental triggers that induce blooms and toxin production.

The effects of variable concentrations of nitrate or nitrite (the most toxic form) on the survival and growth of dinoflagellates are not well known, and the recommended range of levels for these chemicals are broad (*9–11*). At this point in our understanding of the interaction between TPCs and the environment, nitrate and nitrite levels should be periodically monitored and reported.

In its nonionized form in concentrations >0.5 ppm, ammonia is very toxic to fish (*12*). Maintaining low ammonia concentrations in a tank typically requires extensive biofiltration systems. Biofiltration involves removing ammonia by aerobic bacteriological nitrification. Because high ammonia levels could cause false-positive results (i.e., fish death) in the fish bioassay, ammonia concentrations should be monitored.

Dissolved oxygen levels should be >5 ppm to maintain healthy fish (*12*). Ideally, DO levels should be measured and recorded twice daily and whenever a change in fish health (e.g., abnormal behavior, lesion, or death) is noted.

The pH for bioassays and for culturing *P. piscicida* and TPC species in the laboratory should be in the optimum physiological range for fish (i.e., pH = 7.8–8.4) (*12*). Values outside the physiologically appropriate range induce stress in the fish and may compromise experimental results.

All workshop participants agreed that using antibiotics in the fish bioassay or toxin-induction experiments is not recommended to control populations of pathogenic organisms in the tanks. It is possible that bacteria in the water or associated with the fish are important in triggering toxin production or predatory activity in the TPC species. If experimental fish need to be treated with antibiotics, a prophylactic formalin bath (200 ppm in Instant Ocean may be used 2 weeks before initiating the bioassay. It is possible, however, that pharmacologically active chemicals from manufactured sources may be present in the estuarine environment. Theoretically, that material could play a role in the unintentional selection of organisms resistant to pharmacological agents in the environment. Therefore, if any antibiotics are used in an experimental system either for treating fish or research purposes, their effects should be recorded.

Biological Parameters

Table 4 lists the biological parameters that should be reported with experimental

Table 4. Biological parameters for conducting fish bioassays and toxin induction experiments applicable to research involving *P. piscicida* and TPC organisms and the toxins they produce.[a]

Biological parameter	Specification or recommended range of values
Seed culture[b]	200 cells/mL (final culture concentration)
Fish species[c]	Tilapia (*T. mosambica, T. zilli, O. niloticus, O. aurea*), mummichog, menhaden, sheepshead minnow, bullhead minnow, zebrafish *S. galilaeus*
Fish age	Juvenile, nonreproductive
Fish sex	Male

[a]The parameters presented are those with specifications or recommended ranges of values. [b]The seed culture may be a genetically pure culture of a toxic *Pfiesteria* complex species that has demonstrated the ability to kill fish in a fish bioassay, or an environmental water sample containing suspected TPC organisms. [c]Wild-caught fish should not be used in these assays.

results. Aquaria and culture flasks are dynamic experimental systems that may contain many organisms (e.g., bacteria, diatoms, protozoa, viruses, fungi) in addition to those intentionally added by the investigator, especially if environmental samples are used. Some of these organisms are part of the natural community that occurs in the estuarine environment and may be important in the interactions between fish and *P. piscicida*; therefore, documenting the biological parameters for fish bioassay and toxin induction is critical for interpreting the results of these experiments.

Positive control experiments include organisms that will cause toxicity (*P. piscicida* or TPC species). Negative control experiments are those that include organisms that may be similar to TPC species but do not induce toxicity. The use of positive and negative controls (using organisms that are appropriately identified and characterized) assures the investigator that experiments are operating within optimum conditions and that the results are valid. To interpret study results, investigators must report the specific parameters and outcomes of both positive and negative controls for each fish bioassay or toxin-induction experiment.

Questionnaire respondents reported using an extremely wide range of TPC concentrations (100–10,000 organisms/mL from environmental or laboratory-cultured inoculants) for bioassays. A final system volume of 200 cells/mL was considered by workshop participants to be a reasonable minimum concentration.

Bacteria are part of the community of organisms inhabiting the fish bioassay or toxin-induction experiment. Some of these organisms are an integral and necessary component of the natural community, and some organisms are opportunistic pathogens that infect stressed fish. These organisms may have no effect on the assay environment, or they may prey on TPC species (possibly producing false-negative results) or cause illness in fish (possibly producing false-positive results). Because the role of bacteria in these assays is only vaguely understood, analysis of bacteria in samples should be performed whenever possible and reported (presence/absence of certain organisms). Measuring turbidity in the system may also be useful as an indication of bacterial loading.

Wild-caught fish can introduce unknown parasites and pathogens and should probably not be used for these assays and experiments. Tilapia are easy to maintain and are resistant to poor water quality. Because several closely related cichlid fish species are collectively referred to as "tilapia," the exact species should be mentioned (e.g., *Tilapia mosambica, Tilipia zilli, Oreochromis niloticus, Oreochromis aurea, Sarotherodon galilaeus*). One limitation to using tilapia is that it may not be possible to generalize results from experiments using these fish to other species, such as native menhaden. Menhaden (*Brevoortia tyrannus*) may be more difficult to rear and maintain in tanks, but an estuarine fish native to ecosystems where *Pfiesteria*-like organisms are found may be most appropriate for use in these experiments.

The age of the fish is unlikely to be important in culturing TPCs specifically to produce toxin. However, small fish are more likely to survive in a laboratory environment, particularly in culture flasks. For both the flask experiments and aquaria, and on the basis of the age of observed species in the wild, participants agreed that fish should be young (i.e., not of reproductive age).

Nutrition can be an important influence on overall fish health as well (*11,13,14*). A standard flake food should be used to feed fish used in these assays. The specific type of food should be documented, it should be stored under conditions that meet the recommendations of the manufacturer, and investigators must be sure that it has not been contaminated. The amount of food (mass of food per gram of body weight) should be recorded and reported.

Additional Parameters

The parameters discussed above are experimental factors likely to influence the outcome of fish bioassays and toxin-induction experiments and thus should either be limited to a specific range of values or documented and reported by investigators. Many other areas, including good laboratory practices, the use of environmental samples, and fish histopathology, that are currently being addressed as research questions could affect the results of these experiments. These topics were discussed by workshop participants and questionnaire respondents and are discussed below.

Good laboratory practice requires using appropriately identified and characterized organisms for the fish bioassay and toxin-induction work. TPC organisms must be appropriately identified (using light microscopy, molecular biology, or scanning electron microscopy techniques) to associate a positive result for a fish bioassay or toxin-induction experiment with the presence of these organisms (*15–17*). The method used to confirm TPC species, as well as the density of cells, should be reported with the results of the experiment.

The volume of water allowed per fish differs substantially among laboratories, even for those using fish of the same species, size, or age. Experimental evidence indicates that toxin production depends on the presence of fish excreta or secreta. The mass of the fish used in an experiment may be a good surrogate measure for the substance(s) to which TPC species respond. For toxin production, the group proposed a guideline of 0.75 g fish/L aquarium water. The fish bioassay can also be conducted using fish larvae, and a recommended experimental ratio of mass to volume is five larvae/2 mL water.

The use of environmental samples to conduct fish bioassays or to produce toxin may introduce factors, in addition to the biological factors discussed above, that can affect the results of these experiments. Depending on the sample being tested, it may be appropriate to analyze the samples for potentially toxic organic chemicals, including polyaromatic hydrocarbons, polychlorinated biphenyls, and persistent pesticides. It may also be appropriate to analyze environmental samples for levels of metals (e.g., iron, copper, selenium) and other nutrients (e.g., phosphate) to determine possible interactions with toxin production or its biological effects.

Histopathologic examination of experimental fish (including intoxicated fish and fish exposed to positive or negative control organisms) is critical to our understanding of the biological activity of these estuarine toxins. It would be useful to remove moribund fish (i.e., fish that are unable to remain upright or display labored respiration, severe disorientation, or insensitivity to stimuli) from the experimental tank or flask rather than waiting until all movement stops. Upon removal from the tank the moribund animal should be killed by cervical dislocation and immediately placed in fixative (typically 100% neutral buffered formalin). Quick removal of dead or moribund fish also decreases possible toxic effects from overgrowth of extra-experimental organisms (e.g., bacteria and fungi). Protocols describing standard methods for fixing, staining, and interpreting tissues for histopathologic examination are available (*18–22*).

The questionnaire and workshop discussion addressed several temporal factors in the design of fish bioassays and toxin-induction experiments, including the amount of time allowed for experimental vessels to equilibrate before adding experimental organisms. Although the specific conditions vary (i.e., use of an ammonia-nitrate bath), most laboratories report conditioning aquarium filters for 3–4 weeks before adding TPC species and test animals. Most laboratories report that fish are added immediately after the TPC sample (i.e., cultured organisms, water, or sediment) to prevent toxic organisms from transforming to nontoxic forms.

A wide range of times for conducting the experiment (7days to several weeks) was reported by the different laboratories. Although this range may reflect various research goals, it also may indicate a need for better definition of decision points for the bioassay (especially when to end the experiment). Longer exposure times increase the probability of deterioration of water quality and the risk that fish may die of exposure to high levels of contaminants such as ammonia. Although the group could not identify a specific period to run the assay, the length of the assay, as well as the environmental parameters of the system, must be monitored and reported.

For toxin production the group also discussed the number of cycles of exposure and fish death required before the TPC organisms are in sufficient concentration that the water can be used to identify and characterize the toxin. Intuitively, one would assume that the number of fish-death cycles in a toxin-induction experiment increases the amount of toxin in a system, provided the material being produced does not rapidly decompose. Too many cycles, however, may increase waste and other products that can interfere with the interpretation of the assays. By contrast, ending the experiment too soon may preclude obtaining sufficient material for chemical isolation and identification or not allow for the recovery of organisms damaged in transport to the laboratory facility. Clearly, many factors must be considered before the optimum length of time for toxin production can be determined.

In general, monitoring of environmental and water-quality parameters requires a balance between recording specific test-system parameters and minimizing risks for contamination or disturbing the experimental system. For example, closed flasks are less subject than open tanks to contamination by airborne molds and bacteria; however, each time a closed flask is opened and instruments introduced to measure environmental parameters or extract samples, contaminant organisms can be introduced to the system. The optimal frequency of measurement will differ with the type of exposure or culture system. For example, in a relatively small volume (e.g., 250 mL) of water, repeated removal of water for testing can exacerbate water-quality problems.

Conclusion

Future research conducted to understand the biology, biochemistry, and molecular biology of *P. piscicida*, other TPC organisms, and any toxins they produce will involve contributions by investigators from many laboratories. These environmental toxins must be identified and characterized so a reliable and effective method for their detection in environmental and human specimens, and perhaps in other organisms (i.e., fish and other mammals), can be developed. Workshop participants agreed that fish bioassays and toxin-induction experiments should be reproducible, both within and among laboratories. Although many questions still exist about the materials and methods associated with these assays, some experimental parameters (e.g., water temperature in the experimental tanks or flasks) should be maintained within specified ranges. Other parameters may be important and should be recorded and reported by investigators, especially if any of the procedures deviate from the normal methods used in the laboratory. When possible, negative control organisms should be run alongside the assays, and if a positive control organism or material becomes available, it should also be included with the other assays. There are gaps in the current knowledge of the biological, biochemical, and physical interactions in estuaries (e.g., the impact of trace metals and other nutrients on the life cycles of TPC organisms) that are reflected in these bioassays, leaving many research questions as yet unanswered. As more is learned about how these parameters affect these complex biological systems, that knowledge needs to be published and shared with the greater scientific community.

Further research is needed before standardization of these assays can occur, and limiting any of these parameters at this point could hinder progress. All workshop participants have agreed, however, that the parameters suggested in this document are starting points for further discussion. Good laboratory practices and documentation and reporting of parameters will clarify the effects of potential confounders that can be present in fish bioassays and toxin induction assays. Physical, chemical, and biological parameters such as those listed in Tables 2–4, should be recorded as part of the daily experimental regimen (or at the frequency suggested in the table). Parameters should be added to or deleted from these tables as new information presents itself to the research community. Continued communication between laboratories and publication of the methods and parameters described in this report will ultimately lead to the development of quality assurance methods with standardized quality control materials for these experiments.

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